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14. ABSTRACT Predisposition to rheumatoid arthritis (RA) is associated with the presence of genetic factors, HLA class II molecules, DR4 and DQ8, being the strongest. Patients with RA show an imbalance of gut microbiota suggesting its role in regulation of disease. Recently, we isolated <i>Prevotella histicola</i> , anaerobic commensal bacteria of Human gut, from bowel of a patient and have shown that it possesses anti-inflammatory activity. We propose that gut microbiota can influence peripheral immune response and may modulate arthritis in a murine model. HLA transgenic mice expressing RA-susceptible genes DR4 and DQ8 develop collagen-induced arthritis (CIA) following immunization with type II collagen (CII). We have used HLA-DR4/ DQ8 mice to test our hypothesis that treatment with commensal bacteria like <i>Prevotella histicola</i> can modulate CIA. First using various doses of bacteria, we have identified the optimal dose for mice and then used that dose for treatment of arthritis. Treatment of mice with <i>P. histicola</i> as probiotics and therapy are ongoing. In vitro study showed that treatment of mice with <i>P. histicola</i> in CII-immunized mice led to suppression of antigen-specific immune response and reduction in production of inflammatory cytokines. Our data suggests that <i>P histicola</i> induced immune responses in the gut can induce tolerance in periphery leading to systemic immune suppression.					
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Progress Report

This progress report is from April 15, 2010 to Jan 31, 2011.

Introduction

Rheumatoid arthritis is associated with the presence of certain MHC alleles, HLA-DR4 and DQ8 offering the strongest association (1, 2). Analysis of fecal microbiota of patients with RA showed significantly less *Bifidobacteria* and bacteria of the *Bacteroides-Porphyromonas-Prevotella* group, *B. fragilis* subgroup, and the *E. rectale* – *C. coccoides* group than the fecal microbiota of patients with non-inflammatory Fibromyalgia (3). Since these bacterial species are known to belong to the most common genera and groups in the human fecal microbiota, their absence in RA patients might suggest a protective role of these commensal bacteria in RA. Using mice expressing HLA-DR4 and DQ8, we generated a model of collagen-induced arthritis (CIA) that mimics human rheumatoid arthritis in autoantibody profile and sex-bias (4-6). In this proposal we aim to investigate immunomodulatory properties of *P. histicola* to suppress inflammatory autoimmune diseases in HLA-class II Tg mice. The major goal of these experiments is to determine if mucosal immune tolerance is a viable option for the treatment of rheumatoid arthritis.

Our preliminary data showed that HLA-DQ8 mice immunized with type II collagen and fed with *P. histicola* develop much lower incidence of arthritis suggesting immunosuppressive properties of *P. histicola*. Since oral treatment with *P. histicola* led to suppression of systemic immune response, it shows that interaction of commensal bacteria at mucosal surfaces can modulate immune response in periphery.

Progress Report

Prevotella histicola was cultured under anaerobic conditions. Briefly, an isolate of *Prevotella histicola*, stored at -70°C in skim milk, is inoculated onto a CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) (Becton, Dickson and Company, Sparks, MD) and incubated anaerobically in an anaerobic jar with AnaeroPack[®] system (Mitsubishi Gas Chemical America, Inc., New York, NY) and incubated at 37°C for 2-3 days. The bacterium is then swabbed into 10 mL of Trypticase soy broth and is anaerobically incubated for 2 days.

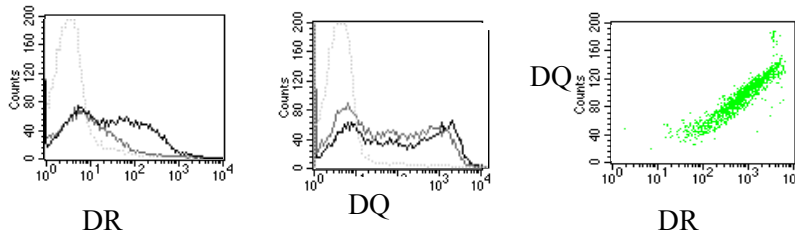


Fig 1. Expression of HLA-DR4 and DQ8 in transgenic mice was analyzed by Flow cytometry in cells isolated from spleen after staining with conjugated antibodies. Expression of DR4 and DQ8 of AE⁰.DRB1*0401.DQ8 mice shows more number of cells expressing the DR and DQ transgene compared to single transgenic mice, Gray line- DRB1*0401.DQ8, Black line- DRB1*0401 mice in DR (left) and DQ8 mice in DQ (middle). Right, B cells express both DQ and DR on the same cell.

mice while littermates were used as controls.

Characterization of transgenic mice: During this phase, we generated transgenic mice expressing HLA-DR4 and DQ8 and extended our studies to double transgenic mice. First, all transgenic mice used *in vivo* and *in vitro* experiments were characterized for the presence of HLA genes by flow cytometry (Fig 1). Only mice positive for the transgene were used as test

Modulation of arthritis in preventive protocol: We immunized mice with type II collagen (CII) to induce arthritis and then fed them *P. histicola* on alternate days 2 weeks following immunization. Next we tested mice in preventive protocol. Mice were fed *P. histicola* 12 days prior to immunization with type II collagen. Mice immunized with type II collagen and fed media without bacteria as well as mice fed bacteria without CII immunization were used as controls. Disease phenotype in CIA model is characterized by paw swelling, scored on scale of 0-3. The studies are in progress and mice are being monitored for development of arthritis. Sera and paws will be collected at the termination of experiments.

***Prevotella histicola* modulates antigens-specific responses:** Since collagen specific T-cell responses play an important role in disease pathogenesis of CIA, we investigated effect of *Prevotella* on antigen specific immune response and production of pro-inflammatory cytokines by antigen specific T-cells. Mice were fed bacteria before or after immunization with CII. Mice immunized with but no bacteria and mice fed bacteria without CII-immunization were used as controls. As shown below in Fig 2, antigen-specific T cell response was suppressed in mice fed *P. histicola* before and after immunization with CII as compared to mice immunized with CII only. As expected, mice fed bacteria in the absence of CII-immunization did not show any antigen specific response. We further tested and compared production of pro-inflammatory cytokines in mice immunized with CII and fed medium and mice immunized with CII and fed *p. histicola* (Fig 3). Mice treated with bacteria after CII-immunization showed a much lower production of proinflammatory Th1 (IL-1, TNF and IFN) as well as Th17 (IL-12(p40), IL-17, IL-6) cytokines compared to mice immunized with CII and fed media without bacteria. These in vitro studies clearly show an immunomodulatory role of commensal bacteria like *P. histicola*. Our studies suggest that *P. histicola* may be able to generate systemic suppression via mucosal immune regulation.

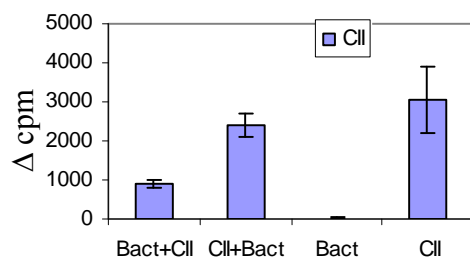


Fig 2 Lymph node T cell response to type II collagen in DR4/DQ8 transgenic mice fed *P. histicola* before and after CII-immunization and control CII-immunized without bacteria and only bacteria fed mice.

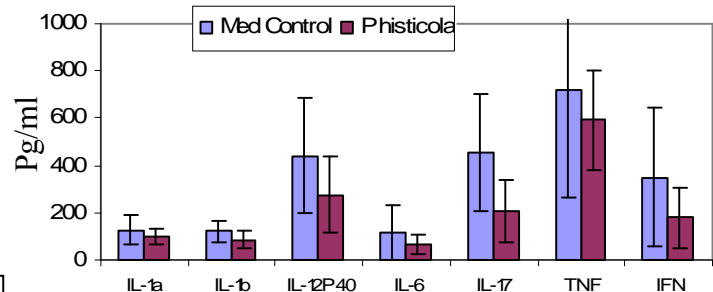


Fig 3 Cytokine production analyzed by ELISA in supernatants of mice immunized with CII and fed media (Med Control) or *P. histicola*. Transgenic mice fed *P. histicola* show a much lower production of proinflammatory cytokines.

Key accomplishments as stated in SOW

Milestone 1# Culture of *P. Histicola*

Milestone#2 Approval of animal protocol

Milestone #3 Generation of DR4/DQ8 transgenic mice for in vivo use. HLA-DR4 and HLA-DQ8 transgenic mice are mated to generate double transgenic mice. Double transgenic mice are characterized for the presence of HLA transgenes by flow cytometry using specific conjugated antibodies. Mice positive for both genes are identified and mated. DR4 and DQ8 transgenes can segregate which necessitates typing for the transgene positivity.

Milestone#4 Mice were gavaged with *P. histicola* for 2 weeks and then immunized with type II collagen. In addition, control mice were gavaged with media in which *P. histicola* were cultured and immunized with type II collagen. Mice are being monitored for arthritis.

Milestone#5 Sera from all test and control mice is being collected and will be used to study antibodies at the termination of the experiment.

Milestone #6 Arthritis is being monitored.

Milestone #7 In vitro experiments show that feeding bacteria suppressed antigen-specific T cell response and reduced production of inflammatory cytokines.

The data obtained from these experiments has been submitted as an abstract to be presented at “Microbiota and Mucosal Immunology Meeting: Interface in health and disease” San Francisco, April 14, 2011.

Conclusions. Our in vitro data showing suppression of antigen-specific immune response in *P. histicola* treated mice suggests generation of peripheral tolerance via gut. Our in vivo ongoing studies may show if antigen-specific tolerance can be used for treatment of arthritis in humanized mice.

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Microbial mucosal modulation of arthritis

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Predisposition to rheumatoid arthritis (RA) is associated with the presence of genetic factors, HLA class II molecules, DR4 and DQ8, being the strongest. Recent reports that patients with RA have decreased fecal levels of certain commensal bacteria suggested that intestinal microbes might be critical in regulation of disease. We isolated *Prevotella histicola*, anaerobic commensal bacteria of Human gut, from bowel of a patient and have shown that it possesses anti-inflammatory activity. We propose that gut microbiota can influence peripheral immune response and may modulate arthritis in a murine model. We have established a murine model of rheumatoid arthritis using mice expressing RA-associated HLA genes, DRB1*0401 and DQ8. DR4 and DQ8 mice develop collagen-induced arthritis (CIA) following immunization with type II collagen (CII). We have used HLA-DR4/ DQ8 mice to test our hypothesis that treatment with commensal bacteria like *Prevotella histicola* can modulate CIA. In vitro data showed that treatment of mice with *P. histicola* in CII-immunized mice led to suppression of antigen-specific immune response and reduction in production of inflammatory cytokines suggesting *P. histicola* has anti-inflammatory properties in this model. Treatment of CIA in transgenic mice in therapeutic protocol is ongoing. Our data suggests that *P. histicola* induced immune responses in the gut causes systemic immune suppression and may be able to regulate autoimmunity.